DELIVERY OF DNA OR RNA VIA GAP JUNCTIONS FROM HOST CELLS TO TARGET CELLS AND A CELL-BASED DELIVERY SYSTEM FOR ANTISENSE OR siRNA

This application claims the benefit of, and priority from, U.S. Provisional Application No. 60/530,555, filed December 17, 2003.

The invention disclosed herein was made at least in part with funding by the U.S. Government, specifically the NHLBI, and NIH(GMS) under grant numbers HL-28958 and GM-55263, respectively, Therefore, the U.S. Government has certain rights in this invention.

Background of the Invention

Throughout this application, various publications are referenced within footnotes or in the text within parentheses. These publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citations for these references may be found at the end of the specification, preceding the claims.

As described in commonly owned prior application U.S. Serial No. 10/342,506, filed January 15, 2003, and in publications (1,2) incorporated by reference herein, stem cells have been used to form gap junctions with target tissues. Such stem cells can influence the activity of the target tissues by delivering gene products or small molecules. However, nucleotides in the form of antisense RNA, or DNA, have not been delivered by host cells (such as human mesenchymal stem cells (hMSCs)) to target tissues.

Summary of the Invention

According to the present invention, RNA can be passed through gap junctions so that engineered cells can be used to deliver RNA to target cells.

According to the present invention, oligonucleotides, either single and double stranded, can be passed through gap junctions formed by C x 43 in HELA cell pairs, as demonstrated by a single electrode delivery of fluorescent-tagged oligonucleotides to a donor cell and determining their transfer to the target cell via gap junction mediated communication. Accordingly, the invention provides for delivery of oligonucleotides to target cells using any donor cell that forms gap junctions.

According to the invention, a method of delivering an oligonucleotide or a plasmid expressing an oligonucleotide into a target cell is provided, comprising introducing an oligonucleotide into a donor cell, and contacting the target cell with the donor cell under conditions permitting the donor cell to form a gap junction with the target cell, whereby the oligonucleotide or a product of the oligonucleotide is delivered into the target cell from the donor cell.

According to the present invention, a method of delivering an oligonucleotide into a target cell is provided, comprising introducing an oligonucleotide into a human mesenchymal stem cell or other donor cell, and contacting the target cell with the human mesenchymal stem cell or other donor cell under conditions permitting the donor cell to form a gap junction with the target cell, whereby the oligonucleotide or its peptide product is delivered into the target cell from the

donor cell.

According to the present invention, a method of delivering an oligonucleotide into a syncytial target cell is provided, comprising introducing an oligonucleotide into a donor cell, and contacting the syncytial target cell with the donor cell under conditions permitting the donor cell to form a gap junction with the syncytial target cell, whereby the oligonucleotide is delivered into the syncytial target cell from the donor cell.

According to the present invention, a method of delivering RNA into a target cell is provided, comprising introducing RNA or a plasmid for RNA into a donor cell, and contacting the target cell with the donor cell under conditions permitting the donor cell to form a gap junction with the target cell, whereby the RNA is delivered into the target cell from the donor cell.

According to the present invention, a method of delivering DNA into a target cell is provided, comprising introducing DNA or a plasmid encoding for DNA into a donor cell, and contacting the target cell with the donor cell under conditions permitting the donor cell to form a gap junction with the target cell, whereby the DNA is delivered into the target cell from the donor cell.

The invention provides a useful treatment in which down regulation of gene activity is desirable (e.g., cancer).

As compared to prior methods wherein delivery of RNA or antisense to target cells is done by a naked plasmid, in the present invention the delivery is via cells, and the transfection rate should be much higher.

Description of the Drawings

Figure 1a shows a 12 member single stranded oligonucleotide passing through gap junction channels composed of connexin 43.

Figure 1b shows a 16 member single stranded oligonucleotide passing through gap junction channels composed of connexin 43.

Figure 1c shows a 24 member single stranded oligonucleotide passing through gap junction channels composed of connexin 43.

Figure 1d shows a 24 member double stranded oligonucleotide passing through gap junction channels composed of connexin 43.

Figure 2a shows a summary of the data where the x-axis is the length of the oligonucleotide, and the y-axis is the relative intensity of the fluorescent tag in the recipient cell (the cell on the left in all of the examples of Figure 1) 12 minutes after delivery of the oligonucleotide to the source cell.

Figure 2b is a graphic representation of junctional conductance on the x-axis versus relative intensity of the fluorescent tag on the y-axis.

Description of the Invention

According to the invention, a method of delivering an oligonucleotide or a plasmid expressing an oligonucleotide into a target cell is provided, comprising introducing an oligonucleotide into a donor cell, and contacting the target cell with the donor cell under conditions permitting the donor cell to form a gap junction with the target cell, whereby the oligonucleotide or a product of the oligonucleotide is delivered into the target cell from the donor cell.

The oligonucleotide may be RNA that can traverse the gap junction or be transcribed into a peptide that can traverse the gap junction. The oligonucleotide may be DNA. The oligonucleotide may be an antisense oligonucleotide or a cDNA that produces an antisense oligonucleotide that can traverse the gap junction. The oligonucleotide may be a siRNA oligonucleotide or a cDNA that produces a siRNA oligonucleotide that can traverse the gap junction. The oligonucleotide may be a DNA or RNA that produces a peptide that can traverse the gap junction. The plasmid may encode siRNA. The oligonucleotide may comprise 12-24 members. The donor cell may be a human mesenchymal stem cell. The donor cell may be a cell containing or engineered to contain connexin proteins. The target cell may be a cell comprising a syncytial tissue, which may be a cardiac myocyte, a smooth muscle cell, an epithelial cell, a connective tissue cell, or a syncytial cancer cell. The target call may be a white blood cell.

The gap junction channels may be composed of one or more of connexin 43, connexin 40, connexin 45, connexin 32 and connexin 37.

According to the present invention, a method of delivering an oligonucleotide into a target cell is provided, comprising introducing an oligonucleotide into a human mesenchymal stem cell or other donor cell, and contacting the target cell with the human mesenchymal stem cell or other donor cell under conditions permitting the donor cell to form a gap junction with the target cell, whereby the oligonucleotide or its peptide product is delivered into the target cell from the donor cell.

According to the present invention, a method of delivering an oligonucleotide into a syncytial target cell is provided, comprising introducing an oligonucleotide into a donor cell, and contacting the syncytial target cell with the donor cell under conditions permitting the donor cell to form a gap junction with the syncytial target cell, whereby the oligonucleotide is delivered into the syncytial target cell from the donor cell.

According to the present invention, a method of delivering RNA into a target cell is provided, comprising introducing RNA or a plasmid for RNA into a donor cell, and contacting the target cell with the donor cell under conditions permitting the donor cell to form a gap junction with the target cell, whereby the RNA is delivered into the target cell from the donor cell.

According to the present invention, a method of delivering DNA into a target cell is provided, comprising introducing DNA or a plasmid encoding for DNA into a donor cell, and contacting the target cell with the donor cell under conditions permitting the donor cell to form a gap junction with the target cell, whereby the DNA is delivered into the target cell from the donor cell.

The present invention provides a way to pass oligonucleotides (DNA and/or RNA fragments) through gap junction channels. This has been demonstrated in experiments where gap junction channels composed of connexin43 (Cx43) were used in a HeLa cell line.

The experiments determined that oligocomplexes such as DNA or RNA sequences of defined length are able to pass through a gap junction channel. DNA or RNA forms alpha helixes in solution with minor diameters of 0.9-1.0 nm. Oligonucleotides in the 12-24 member size range are of particular interest. Unique sequences of DNA which could not be broken down into smaller fragments were tagged with a fluorescent probe from Morpholino, a company which specializes in the manufacture of oligo sequences.

The experiments were conducted with a 12 member oligonucleotide, a 16 member oligonucleotide and a 24 member oligonucleotide. The results demonstrated that all three single stranded forms pass through gap junction channels composed of Cx43 (Figure 1a, b, and c). Further, two 12 member compliments were hybridized producing a double stranded form and its passage was measured (Figure 1d). The double stranded version has only a small increase in its minor diameter.

Figure 2A shows a summary of the data where the X-axis is the length of the oligonucleotide. The hybridized 12 member oligonucleotide is plotted out of sequence on the X-axis. The Y-axis is the relative intensity of the fluorescent tag in the recipient cell (the cell on the left in all of the examples of Figure 1) 12 minutes after delivery of the oligonucleotide to the source cell. For each oligonucleotide the individual experimentally derived values are shown along with the mean

and standard deviation for each oligonucleotide. In a number of experiments junctional conductance and the transfer of fluorescently labeled oligonucleotide were monitored simultaneously.

Figure 2B is a graphic representation of junctional conductance on the X-axis versus relative intensity of the fluorescent tag on the Y-axis. For comparison the conductance-intensity relationship for Lucifer Yellow passage through Cx43 gap junction channels is shown (Valiunas et al., 2002)(2). In all cases the relative intensity, which represents the transfer rate from one cell to another, is 5-10 times less than the Lucifer Yellow fluorescence intensity in recipient cells. This lower transfer rate is consistent with the rod-like dimensions of the oligonucleotide, whose minor diameter is 1.0 nm, being less mobile in solution than Lucifer Yellow.

These observations demonstrate that gap junction channels are a feasible delivery port for molecules such as silencing RNA (siRNA) or any other molecule of similar dimension.

We have previously demonstrated that hMSCs make gap junctions with each other and target cells. We have also demonstrated previously that one can load plasmids into stem cells by electroporation. The present results demonstrate that any donor cell type which forms gap junctions with another target cell type (this includes hMSCs as potential donor or target cells) can be used as a vehicle to deliver RNA or DNA.

References

1. Plotnikov AN, Shlapakova IN, Danilo P Jr, Herron A,Potapova I, Lu Z, Valiunas V, Doronin S, Brink PR, Robinson RB, Cohen IS, Rosen MR: Human mesenchymal stem cells transfected with HCN2 as a gene delivery system to induce pacemaker function in canine heart. Circulation 108: IV-547, 2003.

2. Valiunas et al., 2002 Cardiac gap junction channels show quantitative differences in selectivity. Cir. Res. 91:104-111